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NCBI Coffee Break

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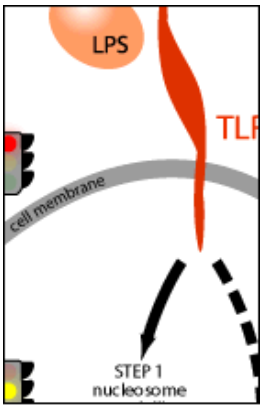
Coffee Break archives

nature REVIEWS Story by Elaine Bell, Nature Reviews Molecular Cell Biology



TLR4 signalling pathway. Transcription of IL-12 p40 is activated by coordinated steps leading from the TLR4 receptor.

Click on the figure for more information.

Ready, steady, go!

Traffic lights regulate the movement of vehicles on roads by transmitting 'stop', 'get ready' and 'go' signals to drivers. Similarly, antigen-presenting cells use cytokines as stop and go signals for lymphocytes. But what's the switch that changes the signal? In the January issue of *Nature Immunology,* Amy Weinmann and colleagues describe a two-part switch for regulating transcription of a cytokine gene: one signalling pathway leads to chromatin remodelling, and a second, independent pathway activates transcription.

An important element in the initiation of inflammatory responses is the activation of macrophages, resulting in the production of pro-inflammatory cytokines such as interleukin 12 (IL-12), a heterodimeric protein comprising p40 and p35 subunits. Toll-like receptors (TLRs), which are expressed on macrophages, recognize microbial molecules and transmit signals that initiate transcription of cytokine genes; TLR4 recognizes the Gram-negative bacterial product lipopolysaccharide (LPS). TLRs use several signalling pathways, including the nuclear factor κB (NF-κB) and Jun N-terminal kinase pathways, to initiate gene transcription. Which of these pathways stimulates macrophages to produce IL-12?

Using restriction enzyme accessibility assays, Weinmann and colleagues found that TLR4 signalling in response to LPS activation results in nucleosome remodelling at the p40 promoter. Curiously, although active NF-κB is essential for transcription of p40, remodelling was not dependent on NF-κB or another transcription factor, CCAAT enhancer-binding protein β. It seems that other TLR4-inducible factors can stimulate remodelling, perhaps making the p40 promoter more accessible to transcription factors such as NF-κB.

So chromatin remodelling a previously unrecognized endpoint of TLR signalling behaves like an amber signal that prepares the chromatin for NF-κB, the green light for transcription of p40. But what is the identity of the protein that recruits the remodelling complex, and what exactly is this complex? Further work in this area should enhance our understanding of TLR signalling and the regulatory mechanisms controlling induction of the inflammatory response.

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Map Viewer tutorial
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Ready, steady, go!

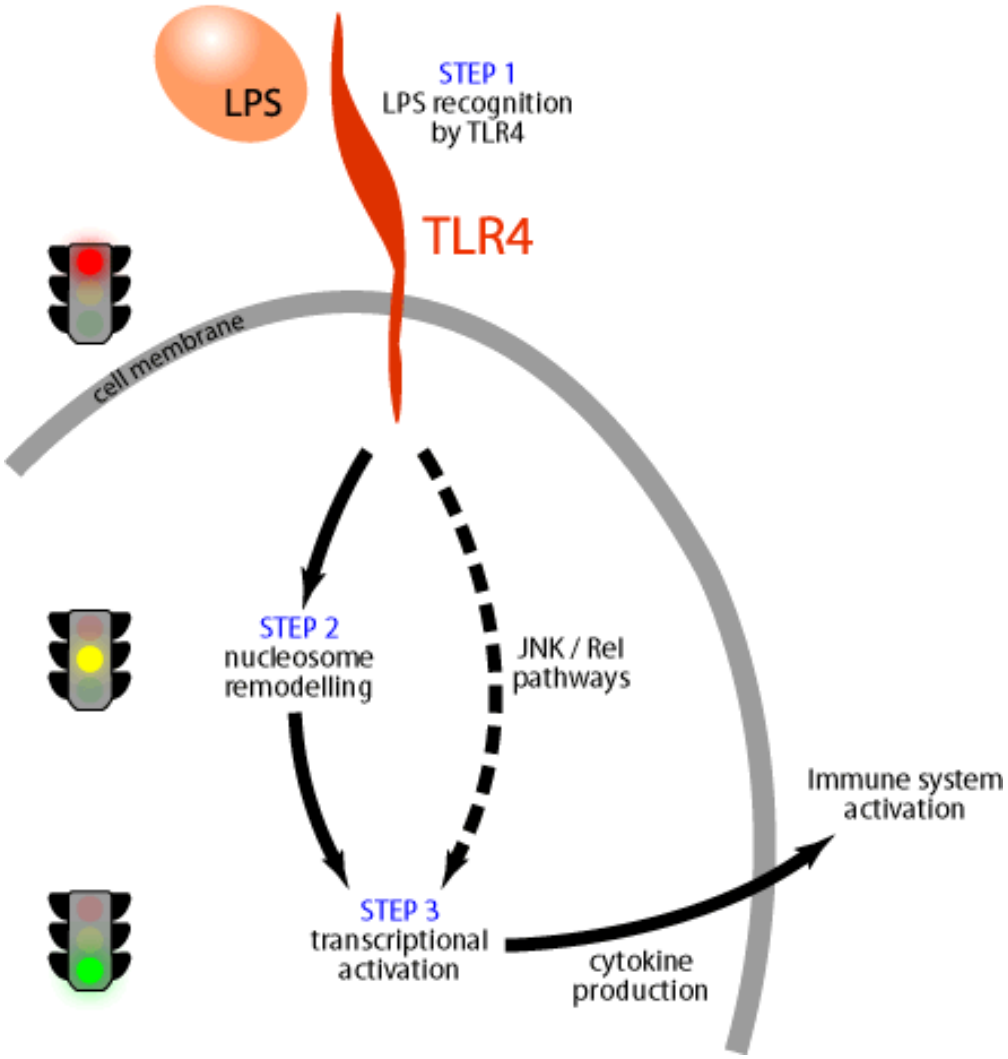

Figure 1. TLR4 signalling pathway. Activation of IL-12 subunit p40 transcription involves several coordinated steps. In the first step, Toll-like receptor 4 (TLR4) recognizes the bacterial surface molecule lipopolysaccharide (LPS). TLR4 then triggers the p40 nucleosome to undergo a remodelling event that is directed by an as yet unknown factor. TLR4 also activates other pathways such as Rel and JNK which act upon the remodelled nucleosome in the third step. This series of events provides a "green light" that allows the transcription of the IL-12 p40 gene to take place. The cytokine IL-12 is an important regulator of immune functions such as inflammation and Th1 development.

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PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Help

Search for [ ] on chromosome(s) [ ] Find

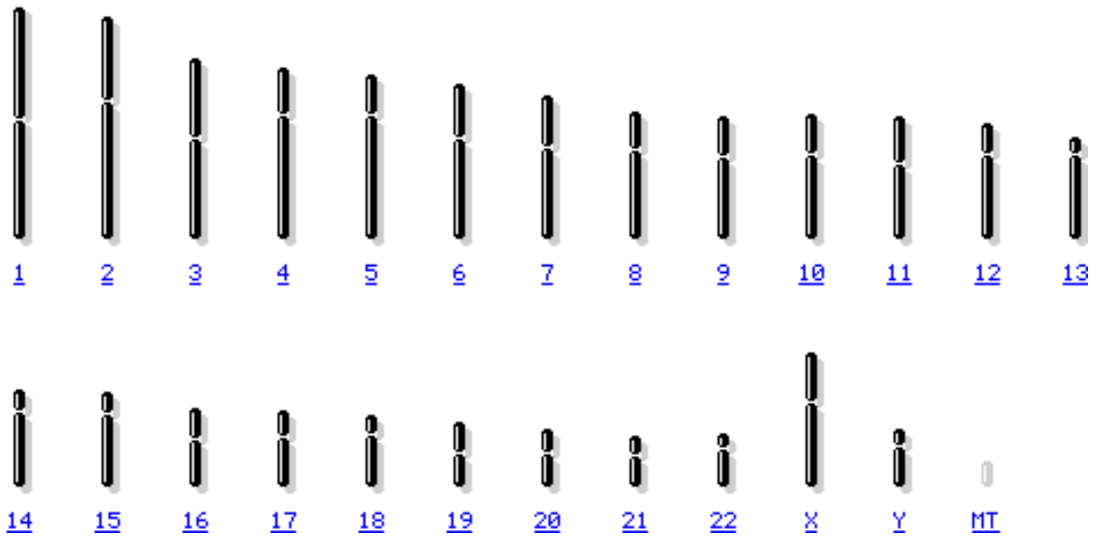
Show Variation (SNP) results Help FTP

The authors of the original research article in this issue of Coffee Break used a mouse model to study TLR4 signalling. Is there a correlating receptor in the human genome? One way to find out is to do a Blast search of the human genome. This type of query will search for sequences similar to mouse TLR4 in the human genome.

To begin a BLAST search of the draft human genome sequence, click on the link marked by the red arrow below. (Note: selecting other links will take you out of this tutorial.)

Homo sapiens **genome view**

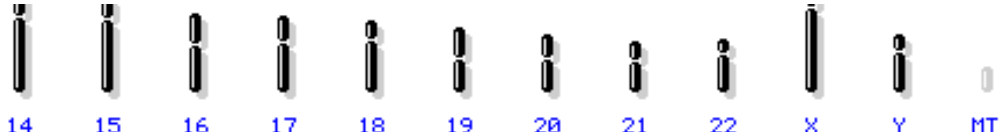
BLAST search the human genome



The NCBI Map Viewer presents a graphical view of the available Human Genome sequence data as well as cytogenetic, genetic, physical, and radiation hybrid maps.

The Map Viewer provides displays of the Human Genome sequence for the NCBI contigs (the 'Contig' map; see assembly description), the BAC tiling path (the 'GenBank' map), and the location of genes, STSs, FISH mapped clones, and variation on the contig sequence.

You can find genes or markers of interest by submitting a query against the whole genome, or a chromosome



ormat. The
en in the
me link in the

Additional information on display control, navigation, and zoom control for the MapViewer is available in the **Help document**; descriptions of the **human maps** displayed are also provided. A separate document provides more detail about the status of the **human genome sequence data**.

NCBI

Tutorial Coffee Break

Chrs: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

Search Contigs for Go

Human Genome Sequencing

BLAST Home Page

BLAST overview

BLAST FAQs

BLAST news

BLAST manual

BLAST the Human genome

The query sequence is submitted on this page. The accession number of mouse TLR4 (NM_021297) has been entered as the query sequence in the box below. Click the "Begin Search" button to continue.

Compare your query sequence to the working draft sequence of the human genome or its mRNA and protein products.

Database: genome Program: blastn Begin Search

Enter an accession, gi, or a sequence in FASTA format:

NM_021297

Optional parameters

Expect	Filter	Descriptions	Alignments
0.01	default	100	100

Advanced options:

Begin Search Clear Input

Questions or Comments?
Write to the NCBI Service Desk

After a request for a BLAST search has been successfully submitted, a "Request ID" (RID) is assigned. The estimated time to process the job is listed below. Most RIDs are stored for up to 24 hours, allowing users to check back at their convenience. Click on the Format! button to see the results of the BLAST search.

Your request has been successfully submitted and put into the Blast Queue.

Query = gi|10946593|ref|NM_021297.1| Mus musculus toll-like receptor 4 (Tlr4), mRNA (3866 letters)

The request ID is 983895091-14596-21247

Format! or Reset all

The results are estimated to be ready in 39 seconds but may be done sooner.

Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

Format

Show Graphical Overview NCBI-gi Alignment in HTML format

Number of: Descriptions 100 Alignments 100

Alignment view Pairwise

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The BLAST search has resulted in 2 hits on the query sequence. The pair-wise alignments of these matches can be viewed below. Click on the "Genome View" button to see the distribution of the BLAST hits in the human genome.

BLASTN 2.1.2 [Nov-13-2000]

Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

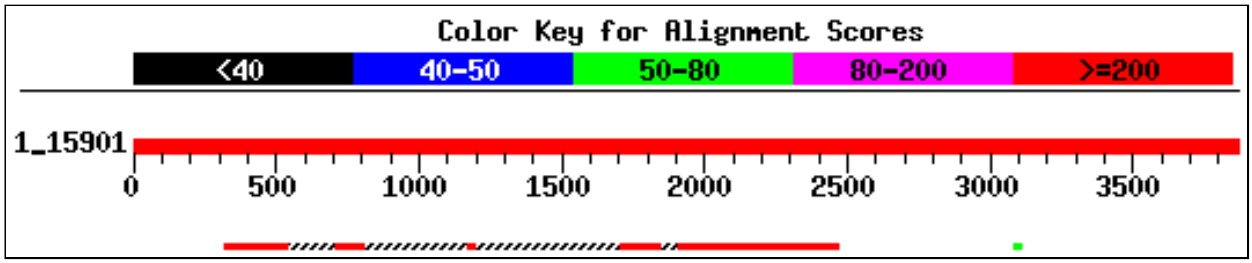
RID: 983895091-14596-21247

Database: Homo sapiens genomic contig sequences
5499 sequences; 2,872,299,031 total letters

Genome View ← Show positions of the BLAST hits in the human genome using the Entrez Genomes MapViewer

Query= gi|10946593|ref|NM_021297.1| Mus musculus toll-like receptor 4 (Tlr4), mRNA
(3866 letters)

Distribution of 6 Blast Hits on the Query Sequence



Sequences producing significant alignments:		Score (bits)	E Value
gi|12734586|ref|NT_017568.2|Hs9_17724	Homo sapiens chromoso...	442	e-121
gi|12729053|ref|NT_022407.2|Hs3_22563	Homo sapiens chromoso...	50	0.009

Alignments

>gi|12734586|ref|NT_017568.2|Hs9_17724 Homo sapiens chromosome 9 working draft sequence segment
Length = 4809520

Score =
442 bits (223), Expect = e-121
Identities = 478/563 (84%)
Strand = Plus / Minus

Query: 1906 aagacaatcatcagtgtgtcagtggtcagtgtgattgtggtatccactgtagcatttctg 1965

```
            ||||| |||||  ||||||| ||  |||||||| |||| |||||   ||||||| |||||
Sbjct: 850311 aagaccatcattggtgtgtcggtcctcagtgtgcttgtagtatctgttgtagcagttctg 850252


Query: 1966   atataccacttctattttcacctgatacttattgctggctgtaaaaagtacagcagagga 2025
            | ||  | |||||||||||||||| ||| |||||||||| | ||||||  | |||||
Sbjct: 850251 gtctataagttctattttcacctgatgcttcttgctggctgcataaagtatggtagaggt 850192


Query: 2026   gaaagcatctatgatgcatttgtgatctactcgagtcagaatgaggactgggtgagaaat 2085
            |||| |||||||||||| ||||| |||||||| || ||| |||||||||||||| || |||
Sbjct: 850191 gaaaacatctatgatgcctttgttatctactcaagccaggatgaggactgggtaaggaat 850132


Query: 2086   gagctggtaaagaatttagaagaaggagtgccccgctttcacctctgccttcactacaga 2145
            ||||| |||||||||||||||||| ||||| |  ||||| ||||||||||||||||||
Sbjct: 850131 gagctagtaaagaatttagaagaaggggtgcctccatttcagctctgccttcactacaga 850072


Query: 2146   gactttattcctggtgtagccattgctgccaacatcatccaggaaggcttccacaagagc 2205
            |||||||||| ||||| ||||||||||||||||||||| ||||| ||||| || |||
Sbjct: 850071 gactttattcccggtgtggccattgctgccaacatcatccatgaaggtttccataaaagc 850012


Query: 2206   cggaaggttattgtggtagtgtctagacactttattcagagccgttggtgtatctttgaa 2265
            || ||||| ||||| || |||||    ||||| || |||||||| ||||||||||||||
Sbjct: 850011 cgaaaggtgattgttgtggtgtcccagcacttcatccagagccgctggtgtatctttgaa 849952


Query: 2266   tatgagattgctcaaacatggcagtttctgagcagccgctctggcatcatcttcattgtc 2325
            |||||||||||||| || |||||||||||||||| ||  |||| ||||||||||||||||
Sbjct: 849951 tatgagattgctcagacctggcagtttctgagcagtcgtgctggtatcatcttcattgtc 849892


Query: 2326   cttgagaaggttgagaagtccctgctgaggcagcaggtggaattgtatcgccttcttagc 2385
            ||  ||||||| |||||| |||||||| ||||||||||||||  |||| |||||||| |||
Sbjct: 849891 ctgcagaaggtggagaagaccctgctcaggcagcaggtggagctgtaccgccttctcagc 849832


Query: 2386   agaaacacctacctggaatgggaggacaatcctctggggaggcacatcttctggagaaga 2445
            || ||||| |||||||| |||||||||| |   |||||| |||||||||||||||||| ||
Sbjct: 849831 aggaacacttacctggagtgggaggacagtgtcctggggcggcacatcttctggagacga 849772


Query: 2446   cttaaaaatgccctattggatgg 2468
            || | ||| |||||  |||||||
Sbjct: 849771 ctcagaaaagccctgctggatgg 849749

 Score =  109
bits (55), Expect = 1e-20
 Identities = 178/219 (81%)
 Strand = Plus / Minus


Query: 324    ccacctctcaaacttgatactgacaggaaaccctatccagagtttttccccaggaagttt 383
            ||||||||| | ||| ||| |||||||||||||| ||||||||||  |||  |||   ||
Sbjct: 851896 ccacctctctaccttaatattgacaggaaaccccatccagagtttagccctgggagcctt 851837


Query: 384    ctctggactaacaagtttagagaatctggtggctgtggagacaaaattggcctctctaga 443
             ||||||||| |||||||| |||| ||||||||||||||||||||  | || ||||||||
Sbjct: 851836 ttctggactatcaagtttacagaagctggtggctgtggagacaaatctagcatctctaga 851777


Query: 444    aagcttccctattggacagcttataaccttaaagaaactcaatgtggctcacaattttat 503
             | |||||| |||||||| || | ||| || ||  |||| |||||||||||||| ||||
Sbjct: 851776 gaacttccccattggacatctcaaaactttgaaagaacttaatgtggctcacaatcttat 851717


Query: 504    acattcctgtaagttacctgcatatttttccaatctgac 542
             || || |  || |||||||  |||||||| ||||||||
Sbjct: 851716 ccaatctttcaaattacctgagtatttttctaatctgac 851678
```

```
 Score =
81.8 bits (41), Expect = 3e-12
 Identities = 116/141 (82%)
 Strand = Plus / Minus


Query: 1708   cattttccaaagagtctagccttcttcaatcttactaacaattctgttgcttgtatatgt 1767
              ||||||||||  |||||||| |||||| ||||||||| | |||    |||||||||  |||
Sbjct: 850506 cattttccaagtagtctagctttcttaaatcttactcagaatgactttgcttgtacttgt 850447


Query: 1768   gaacatcagaaattcctgcagtgggtcaaggaacagaagcagttcttggtgaatgttgaa 1827
              ||||| ||||  |||||||| ||| ||||||| |||| |||| |||||||| | ||||||
Sbjct: 850446 gaacaccagagtttcctgcaatggatcaaggaccagaggcagctcttggtggaagttgaa 850387


Query: 1828   caaatgacatgtgcaacacct 1848
              | ||||  ||||||||||||
Sbjct: 850386 cgaatggaatgtgcaacacct 850366

 Score = 56.0
bits (28), Expect = 2e-04
 Identities = 82/100 (82%)
 Strand = Plus / Minus


Query: 709    ctgactctaagaggtaattttaatagctcaaatataatgaaaacttgccttcaaaacctg 768
              |||||| |||||  ||||||| |||| | |||| ||||||||||||  |||||   |||
Sbjct: 851511 ctgactttaagaaataattttgatagtttaaatgtaatgaaaacttgtattcaaggtctg 851452


Query: 769    gctggtttacacgtccatcggttgatcttgggagaattta 808
              ||||||||| | |||||||| ||| |  ||||||||||||
Sbjct: 851451 gctggtttagaagtccatcgtttggttctgggagaattta 851412

 Score =
50.1 bits (25), Expect = 0.009
 Identities = 28/29 (96%)
 Strand = Plus / Minus


Query: 1170    gagctttagtggttgctgttcttattctg 1198
               |||||||||| ||||||||||||||||||
Sbjct: 4783191 gagctttagttgttgctgttcttattctg 4783163

>gi|12729053|ref|NT_022407.2|Hs3_22563 Homo sapiens chromosome 3 working draft sequence segment
          Length = 285728

 Score =
50.1 bits (25), Expect = 0.009
 Identities = 28/29 (96%)
 Strand = Plus / Minus


Query: 3081   aaatacaattcctagtatacttttacttt 3109
              ||||||| ||||||||||||||||||||
Sbjct: 209235 aaatacatttcctagtatacttttacttt 209207

  Database: Homo sapiens genomic contig sequences
    Posted date:  Feb 22, 2001  5:09 PM
  Number of letters in database: -1,422,668,265
  Number of sequences in database:  5499

Lambda     K      H
    1.37    0.711     1.31

Gapped
Lambda     K      H
    1.37    0.711     1.31
```

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 8780236
Number of Sequences: 5499
Number of extensions: 8780236
Number of successful extensions: 278
Number of sequences better than 1.0e-02: 2
length of query: 3866
length of database: 2,872,299,031
effective HSP length: 22
effective length of query: 3844
effective length of database: 2,872,178,053
effective search space: 11040652435732
effective search space used: 11040652435732
T: 0
A: 0
X1: 6 (11.9 bits)
X2: 15 (29.7 bits)
S1: 12 (24.3 bits)
S2: 25 (50.1 bits)

Ready, steady, go!

Alignment of mouse Tlr4 with human TLR4 on the draft human genome sequence

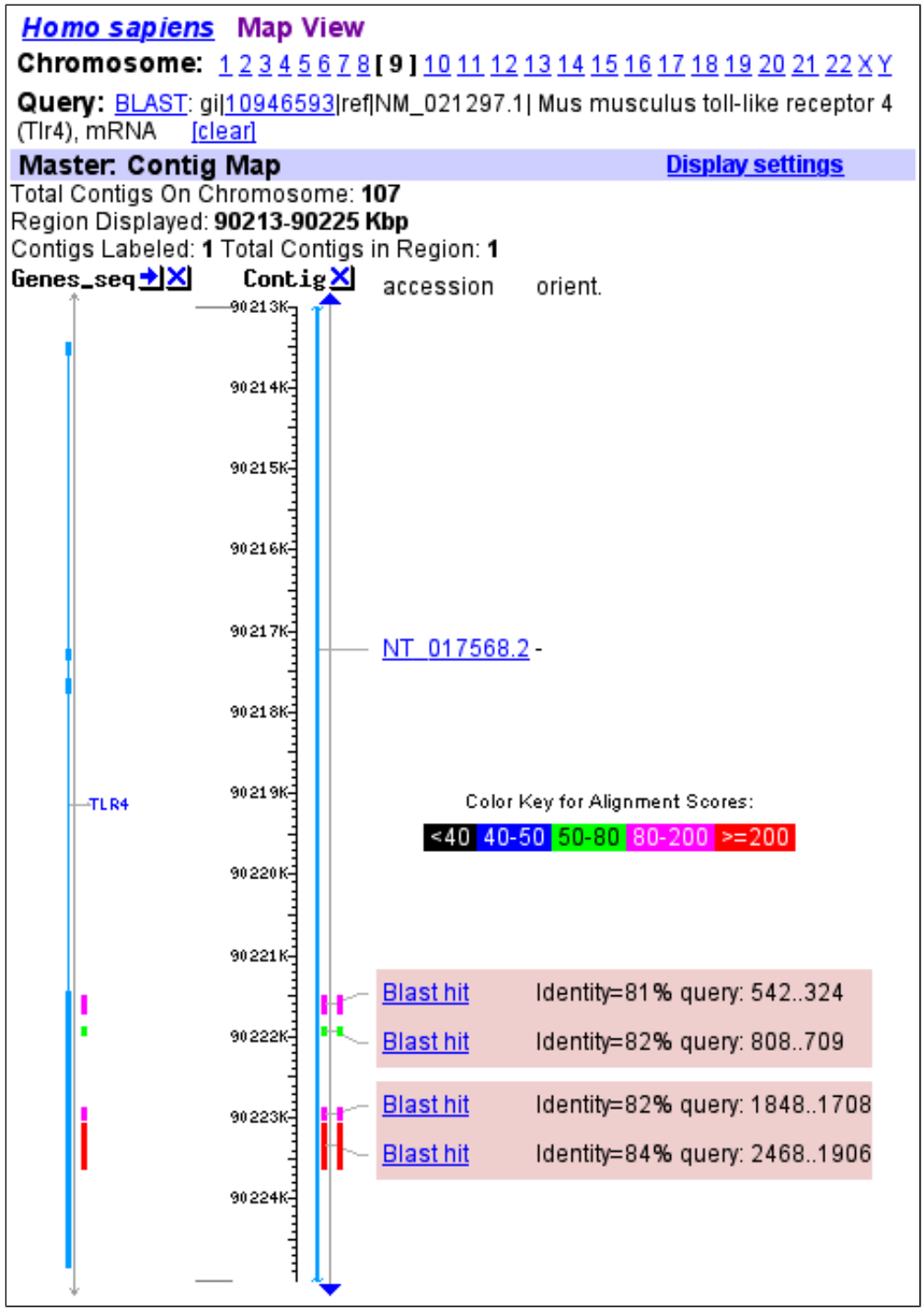


Figure 1

In this Coffee Break tutorial, a BLAST search of the draft human genome sequence was initiated using the mouse *Tlr4* gene (NM_021297) as the query sequence. Figure 1 shows the alignments of the BLAST hits in the human genome on human chromosome 9, using MapViewer. The "Genes_seq" map shows the positions of annotated genes, in this case the human *TLR4* sequence. The "Contig" map shows the contiguous sequence (the actual segment that was assembled into the draft genome) on which the matches are located. Maps may be added or removed by clicking on the Display Settings button in MapViewer. The pair-wise alignment of each BLAST hit may be obtained by clicking on the individual "Blast hit" links (not available in this tutorial).

Toll was originally found in *Drosophila* and was implicated in the formation of dorsoventral polarity and anti-microbial resistance [1, 2]. The Toll protein is a transmembrane protein that consists of extracellular, transmembrane, and cytoplasmic domains [3]. It was discovered that the cytoplasmic signalling domain of Toll has many similarities to the interleukin-1 receptor (IL-1R), which led to the investigation of Toll as an important component in innate immunity [4]. It is now known that there are many forms of Toll, including the human homolog Toll-like receptor (TLR) that stimulates gene expression in response to lipopolysaccharide (LPS) [3]. TLRs control gene expression through a variety of pathways including the NF-κB and JNK pathways [2, 5-7].

Weinmann and colleagues have discovered that signalling through the mouse Toll receptor results in a two-step activation of the gene encoding IL-12 p40 [8]. What remains to be discovered, however, are the elements that recruit a remodeling complex to the p40 promoter. Once this factor is elucidated, it will be interesting to go back and BLAST the human genome to search for an equivalent protein.

Mouse TLR4 (NM_021297) was used to search the draft human genome sequence using the BLASTN program [9] with standard parameters. The BLAST sequence alignment was constructed using MapViewer.

[1] Anderson KV, Jurgens G, and Nusslein-Volhard C. (1985) Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. *Cell* 42, 779-789.

[2] Lemaitre B, et al. (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell* 86, 973-983.

[3] Means TK et al. (2000) Structure and function of Toll-like receptor proteins. *Life Sci* 3, 241-58.

[4] Gay NJ and Keith FJ. (1999) Drosophila Toll and IL-1 receptor. *Nature* 351, 355-356.

[5] Dusahy MS, et al. (1996) Origins of immunity: Relish, a compound Rel-like gene in the antibacterial defense of Drosophila. *Proc Natl Acad Sci USA* 93, 10343-10347.

[6] Wu LP and Anderson KV. (1998) Regulated nuclear import of Rel proteins in the Drosophila immune response. *Nature* 392, 93-97.

[7] Williams MJ, et al. (1997) The 18-wheeler mutation reveals complex antibacterial gene regulation in Drosophila host defense. *EMBO J* 16, 6120-6130.

[8] Weinmann AS, et al. (2001) Nucleosome remodeling at the IL-12 p40 promoter is a TLR-dependent, Rel-independent event. *Nature Immunology* 2, 51-57.

[9] Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389-3402.